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ABSTRACT

One hundred two male mice, Mus musculus, ICR strain, age four weeks, were obtained. Fifty-one mice were pinealectomized and 51 were sham operated. The animals were divided into three photoperiod groups: 1.5L:22.5D, 14L:10D, 24L:0D. The mice were subcutaneously injected daily with 10 ug melatonin or control solution from post-operative day 3 to post-operative day 60. On post-operative day 60 representatives of each photoperiod-surgery-injection regimen were paired with females. The remaining animals were killed and several organs were removed and weighed. Melatonin injections were progonadal in pinealectomized mice and inhibitory in sham mice in several instances. Histological examination of testis sections indicated normal spermatogenesis and the presence of mature sperm. A reproductive study indicated that all mice were capable of siring offspring irrespective of treatment. Doubt is cast on the significance of the pineal to the reproductive competence of male laboratory mice.

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INTRODUCTION

The mammalian pineal gland originates embryonically as an outgrowth of the roof of the diencephalic vesicle. In the adult it has lost all afferent and efferent neural connections, and instead is innervated by post ganglionic neurons (Kappers, 1960). This organ is phylogenetically new, having lost the photoreceptor cells typical of the amphibian pineal and thus the capacity to generate nerve impulses in response to light. Although the histology of the mammalian pineal is compatible with a secretory function (Kelly, 1962), its specific function is unknown. A growing body of evidence suggests, however, that pineal secretions are associated with photoperiodicity in reproduction (Reiter and Sorrentino, 1970; Reiter et al., 1970; Kinson and Peat, 1971; Reiter, 1973).

As a neuroendocrine transducer, the pineal receives neural signals from the eye. Nerve impulses travel from the stimulated retina along the preganglionic neurons to the superior cervical ganglia (Wurtman et al., 1963; Wurtman and Muscowitz, 1977). These nerve impulses terminate directly on pineal cells called pinealocytes. Stimulation of pinealocytes by light inhibits the release of the pineal secretion

product(s). During periods of darkness, the pineal secretes a number of substances, among them melatonin (Collu and Fraschini, 1972).

The pineal has been found to contain large quantities of serotonin and melatonin in addition to the enzyme hydroxyindole-O-methyltransferase (HIOMT). Serotonin is transaminated to N-acetyl serotonin which is methylated by HIOMT to melatonin (Axelrod, 1974). Ralph et al. (1971) have found that HIOMT activity increases in periods of darkness and decreases in light periods, correlating with observed changes in melatonin levels (Axelrod, 1974). Evidence points to melatonin as probably being the pineal product associated with mammalian reproduction.

Female mammals, notably rats and hamsters, have figured prominently in pineal research. Exposure to continuous light increases estrous activity and causes hypertrophy of the uteri and ovaries in these animals (Wurtman et al., 1963; Collu and Fraschini, 1972). Exposure to constant darkness or short photoperiods resulted in decreased incidence of estrous and atrophy of the reproductive organs (Wurtman et al., 1961). Administration of melatonin to females in continuous light decreased the incidence of estrous and decreases in uterine and ovarian weights were observed (Wurtman

et al., 1963; Chu et al., 1964). Effects of continuous darkness on female reproductive organs were abolished after pinealectomy (Wurtman et al., 1963; Reiter et al., 1975). Melatonin given to rats maintained in continuous darkness reversed the progonadal influence of pinealectomy (Reiter et al., 1975). The above studies have convinced a number of researchers, including Reiter (1973), that one of the major functions of the mammalian pineal is to photoperiodically condition the gonads.

Experiments on the pineal in male mammals are sparse compared to the studies on females. Experiments on male hamsters indicate a pineal function previously found in female mammals. Exposure to longer light periods stimulated the reproductive system of male hamsters, rats and mice; whereas exposure to short photoperiod or complete darkness resulted in atrophy of testes and accessory organs in these same animals (Reiter and Fraschini, 1969; Reiter et al., 1970; Reiter et al., 1974). Pinealectomy of male hamsters exposed to short photoperiod resulted in increased testicular weight and accessory organ weights (Reiter et al., 1974). The administration of melatonin to hamsters in constant light was shown to be anti-gonadotrophic (Reiter et al., 1974; Turek, 1977).

While the above studies indicate that the function of the pineal is the same in both sexes of mammals, there are a number of studies which indicate that the males of some species behave differently in this regard. The results of studies of pinealectomy and melatonin administration on male rats and mice are very inconsistent. Pinealectomy was reported to increase testicular and accessory organ weights in rats (Reiter, 1968) and prevented the inhibitory effect of light restriction on rat testes (Sorrentino et al., 1971). Contrastingly, Kinson and Lui (1973) and Reiter et al. (1975) reported that pinealectomy had no effect on reproductive organs of male rats.

The effects of melatonin injections on male rats and mice are even less clear. Present literature contains reports of melatonin exerting progonadal, antigonadal or no effect on the reproductive system of male rats and mice (Motta et al., 1967; Kinson and Peat, 1971; Reiter et al., 1978). Shugart (1980) observed that the sex accessory organ weights of mice that received melatonin and vehicle injections were not significantly different although organ weights of mice that received melatonin were slightly higher.

Ambiguity of results on male mammals, especially mice, prompted the present study. Other researchers

have employed pinealectomy (Vaughan and Reiter, 1971). varied photoperiod and melatonin injections (Houssay et al., 1966), however, none have employed a combination of these factors as was used in the present study. In addition, this investigation, unlike all others, tested the fertility of subjects from each experimental regimen.

MATERIALS AND METHODS

One hundred two, four week old, male mice, Mus musculus, ICR strain, weighing between 10-20 gm were obtained from Flow Laboratories, Dublin, VA in September 1980.

Surgery and Photoperiod Grouping

Fifty-one animals were pinealectomized using the procedure of Hoffman and Reiter (1965). Each animal was anesthetized with sodium pentobarbital (60 mg/ kg body weight) and placed in a head holding device. A dorsal incision was made above the skull and a circular bone plug, 50 mm in diameter, was made at the junction of the parietal and interparietal bones. The bone plug was removed and a pair of jewelers forceps (#5) was inserted through the dura mater approximately 5-10 mm to remove the pineal. After pinealectomy, excess blood was removed, the bone plug was replaced, and covered with a small piece of Gelfoam (Upjohn Co.) to provide a matrix for coagulation. Wound clips were used to close the incision. The remaining fifty-one animals were sham operated using the above procedure except that the pineal was not removed. Six animals died within one day of surgery.

One half of pinealectomized and sham operated

mice received daily subcutaneous injections of 50 ul of 3% ethanol in Locke's physiological solution (ELP: Hoar and Hickman, 1975). The remaining animals received daily subcutaneous injections of 10 ug melatonin in 50 ul of ELP. Ethanol was needed to dissolve the melatonin. Injections were given between 1100 and 1230 hr from post-operative day 4 through post-operative day 60. It has been shown that injections of melatonin near the end of the subjective day were most effective in inhibiting gonadal activity (Reiter et al., 1976).

Groups containing 16 pinealectomized and 16 sham operated mice were placed in different photoperiods. One group was placed in a Bitronette Mark IV growth chamber (Labline, Inc.) under a 24L:0D (24 hr Light: 0 hr Dark) photoperiod. Another group was housed in a Kysor-Scherr Model CEL-36-10 environmental chamber utilizing a 14L:10D photoperiod (lights on from 2230 to 1230 hr). The third group was placed in a Kysor-Scherr Model CEL-36-10 environmental chamber with a 1.5L:22.5D photoperiod (lights on from 1100 to 1230 hr). Temperatures in the environmental chambers were between 23-27^{o} during the entire experiment. Animals were housed two per cage and were fed Purina lab chow and watered ad libitum. Light intensity at cage floor was 45-55 lumens per square foot and provided by

fluorescent light fixtures. Six animals died during the experiment.

Autopsy

On post-operative day 60 two animals were selected at random from each photoperiod-surgical-injection regimen and were placed separately with females (see Reproductive Study, below). The remaining animals were anesthetized with sodium pentobarbital (45 mg/ kg body weight) and injected with 100 ul of 0.001% heparin. Cardiac punctures were performed using a heparinized syringe with a 27½ ga needle. Blood was pooled from several mice and centrifuged at 2000 x g for 15 min at room temperature. Plasma was collected and frozen for testosterone assay. The following organs were removed and weighed: pituitary, ventral prostate, dorsal prostate and testes. Testes were placed in Bouin's fixative for histological preparation.

Reproductive Study

Twenty-four female mice, M. musculus, ICR strain, approximately six weeks of age, were obtained from Flow laboratories, Dublin, Va in October 1980. Two randomly selected males from each photoperiod-surgical-injection regimen were singly paired with females under a 14L:10D photoperiod. On day 10 the males were

removed and females were allowed to gestate. The number of offspring was counted at birth.

Histological Preparation

Testes removed from mice at autopsy on post-operative day 60 were placed in Bouin's fixative for at least three days. Dehydration was accomplished using a series of alcohols (35% to 100%) and xylene. Testes were embedded in Paraplast and sectioned at 8 um using an American Optical "820" Spencer microtome. Testis sections were mounted on glass slides with egg albumin as the adhesive, placed on a 55° heating tray overnight and rehydrated. Staining was accomplished using Delafield's hematoxylin for 23 min. Slides were destained with 0.01 N HCl, blued with $LiCO_3$, dehydrated and mounted in Canadian balsam. Sections were examined microscopically.

Testosterone Assay

Testosterone from pooled plasma samples obtained at autopsy was extracted with 10 ml methylene chloride and washed with 1.0 ml volumes of 0.1N NaOH, 0.1N acetic acid, and deionized water to remove potentially interfering substances. Aqueous phases were aspirated and the organic phase was evaporated under a gentle stream of air. The dried organic phase was reconstituted with isooctane:benzene:methanol (95:5:5).

Testosterone was separated from other components present by column chromatography using lipophilic Sephadex (LH-20-100, Sigma Chemical Co.). Fractions containing testosterone were dried down, reconstituted with absolute ethanol, and aliquots were placed into duplicate tubes and evaporated. Samples were then assayed for testosterone using the radioimmunoassay method of New England Nuclear (1980).

The basic principle of the radioimmunoassay involves the competition between ^{3}H-testosterone and testosterone for a limited number of antibody binding sites. Non-radioactive testosterone from the plasma samples and a fixed amount of ^{3}H-testosterone were allowed to react with a constant amount of antibody. Consequently, a decreasing amount of ^{3}H-testosterone was bound to the antibody. Separation of free testosterone from antibody-bound testosterone was achieved through selective adsorption of free testosterone onto activated charcoal (New England Nuclear, 1980). A Beckman Instruments LS-100C liquid scintillation system was employed in this study to count tritium content of the samples.

As samples are quenched to some extent by various interactions within the scintillation vial, a quenching curve was essential (Long, 1976). A quench curve was

prepared by adding an amount of tritiated material to 10 ml liquid scintillation cocktail (Aquasol, New England Nuclear). Tritium counts were obtained and varied amounts of quenching material (assay buffer) were added to the vials. Tritium counts and external standard ratios (ESR) were determined again. External standard ratio was plotted against percent tritium quenched. On obtaining tritium counts and ESR from testosterone samples, true counts per minute were obtained using the quench curve. Testosterone content of the samples was determined using a testosterone standard curve and the formulae prepared according to New England Nuclear (1980).

Statistical Analysis

A three way analysis of variance (ANOVA) was performed on all organ weights expressed as gram weights and as percent body weight. Percent body weight was used to take into account variances in body weight at the conclusion of the experiment. Analysis of variance was performed using the Statistical Package for the Social Sciences (SPSS, Northwestern University) which showed the presence of any trends and interactions between two or three of the controlling variables in the experiment. Controlling variables were photoperiod, surgery, and injection. When the ANOVA test determined

differences, the differences were detected using Student's t-test.

For all statistical analyses a 95% confidence interval was established. Statistical treatment of testosterone levels and offspring number was not possible due to the necessity of pooling blood samples and the limited number of cases in the reproductive study.

RESULTS

When body weights (both as actual and percent increase) of pinealectomized mice that received vehicle injections were compared with their sham counterparts at the three photoperiods there was a significant difference only at the intermediate (14L: 10D) photoperiod. The actual body weight of the shams were higher than in pinealectomized mice. When comparisons between body weight were made with melatonin injections in the pinealectomized and sham operated mice no significant differences were found. No significant differences were found in body weights among the sham operated mice that received vehicle and melatonin injections at the three photoperiods. A comparison of body weights was made with pinealectomized mice that got vehicle and melatonin injections and showed a significant difference for the intermediate photoperiod. In this case the body weights of mice with vehicle injections were larger than those with melatonin (Table 1; Fig. 1 and 2).

Pituitary weights of pinealectomized mice that received vehicle injections and their controls showed significant differences in each of the three photoperiods. In long and short photoperiods the pituitary

weights (actual and percent body weight) of sham operated mice were greater than those of pinealectomized mice. In the intermediate photoperiod pinealectomized mice had heavier pituitaries than their controls. Among mice that received melatonin significant differences were found in two of three photoperiods. In the short (actual weight) and intermediate (actual and percent body weight) photoperiods the sham operated animals had larger pituitaries than pinealectomized mice. Sham operated mice demonstrated larger pituitaries (actual weight) for melatonin injected mice than for vehicle injected mice in the intermediate photoperiod. Comparisons of pinealectomized mice with melatonin and vehicle injections revealed that vehicle injected mice had larger pituitaries (actual and percent body weight) than did melatonin injected animals in the 14L:10D photoperiod. Melatonin treated mice had larger pituitaries (actual weight) than vehicle injected mice in the long photoperiod (Table 2; Fig. 3 and 4).

Ventral prostate weights of pinealectomized mice with vehicle injections were compared to the sham operated controls with the three photoperiods. Ventral prostate weights among sham controls were larger in the 1.5L:22.5D photoperiod (percent body weight) and in the long photoperiod (actual and percent body weight)

than were those of pinealectomized mice. No significant differences were found among comparisons of pinealectomized and sham operated mice that received melatonin injections. Also, differences were not observed in sham operated mice that received melatonin and vehicle injections with photoperiod. In comparisons of pinealectomized mice that got vehicle and melatonin injections ventral prostate weights (actual and percent body weight) of melatonin injected mice were larger than in vehicle injected mice in the 24L:0D photoperiod. This was also true in the short photoperiod (percent body weight) (Table 3; Fig. 5 and 6).

The dorsal prostate weights (actual and percent body weight) between pinealectomized mice and sham controls that received melatonin injections were significantly different in one of the three photoperiods. Sham operated mice had larger dorsal prostates than pinealectomized mice in the short photoperiod. There were no significant differences between pinealectomized and sham operated mice that received melatonin injections in the three photoperiods. In sham operated mice, vehicle injected animals had larger dorsal prostates (percent body weight) than melatonin injected mice in the long photoperiod. Among pinealectomized mice that were vehicle and melatonin injected

significant differences in two of the three photoperiods were present. In the short and long photoperiods melatonin injected mice had larger dorsal prostates (actual and percent body weight) than the control mice (Table 4; Fig. 7 and 8).

No significant differences occurred in testis weights of pinealectomized and sham operated mice with vehicle injections in the three photoperiods. Among pinealectomized and control mice that received melatonin there was a significant difference in one case. In this instance the sham operated mice had larger testes (actual weight) than did the pinealectomized mice in the long photoperiod. No significant differences occurred in either vehicle injection comparisons or melatonin injection comparisons (Table 5; Fig. 9 and 10).

Histological examination of testis sections revealed no irregularity among different experimental regimens. In all cases seminiferous tubules were well formed, spermiogenesis was taking place and mature spermatozoa were observed (Fig. 12-14).

Testosterone levels of mice exposed to normal photoperiod increased slightly above those mice exposed to a short photoperiod. Increases in testosterone titers were observed in mice exposed to continuous light. Titers were highest in the sham-vehicle regimen

followed by, in decreasing order, sham-melatonin, pinealectomy-melatonin, pinealectomy-vehicle. Statistical analysis was not performed because of the necessity of pooling blood samples which gave only one observation per regimen (Table 6; Fig. 11).

A non-statistical examination of the average number of offspring sired by representatives of each photoperiod-surgical-injection regimen did not show any differences that could be attributed to any controlling variable effects. In Table 7 three cases show the offspring of one representative only. One female did not become pregnant and in two cases the females ate some of their offspring before they could be counted. These cases were omitted from the final results.

DISCUSSION

The fact that most combinations of pinealectomy, melatonin injection and variable photoperiod had no significant effect on body weight in male M. musculus. is in agreement with several studies on male mice (Houssay et al., 1966; Vaughan and Reiter, 1971; Shugart, 1980), male rats (Motta et al., 1967; Debeljuk, 1969; Kinson and Peat, 1971; Kinson and Lui, 1973), and male hamsters (Reiter et al., 1974). Body weight also was not affected by the above variables in the female hamster (Reiter and Hester, 1966; Reiter et al., 1966; Reiter, 1968). Relkin (1972) found that pinealectomy resulted in increased growth hormone levels in the rat using a 14L:10D photoperiod. Sorrentino et al. (1971) and Relkin (1972) have hypothesized that the pineal secretion melatonin inhibits the secretion of growth hormone by acting at the hypothalamic level. What remains unexplained is why this pineal inhibitory influence found consistently by other researchers appears only at the intermediate photoperiod in the present study.

In addition to the influence of the pineal on growth hormone levels, the pineal has also been implicated in the inhibition of follicle stimulating hormone

and lutenizing hormone secretion by the pituitary (Reiter and Fraschini, 1969; Tamarkin et al., 1977; Alonzo et al., 1978). Thus it might be suspected that the pineal would have an inhibitory influence on pituitary weight. The opposite effect was observed, however, as pinealectomy actually reduced pituitary weights and melatonin injections were stimulatory to this organ in the long and short photoperiods. Although this agrees with the study on pinealectomized male mice by Houssay et al. (1966), the present results are opposite those found by Shugart (1980) in melatonin injected mice. Melatonin injections have had no effect on pituitary weights in the male rat (Motta et al., 1967; Kinson and Lui, 1973), male hamster (Reiter et al., 1974), and female hamster (Reiter and Hester, 1966; Reiter and Klein, 1971). Motta et al. (1967) found that pinealectomized female rats had smaller pituitaries. This and other studies suggest that pituitary weight is a poor indicator of pituitary function.

It is well documented that pituitary gonadotrophins stimulate testicular growth and function and many studies in female mammals have found the pineal to be inhibitory to pituitary gonadotrophin release (Turner and Bagnara, 1976). In the present study no overall

effect on testicular weight was found either by pinealectomy or melatonin injection. These findings are in agreement with the results of Vaughan and Reiter (1971) with pinealectomized mice. They are in disagreement with Houssay et al. (1966), however, who found that pinealectomized mice had larger testes than their controls. Reactions of mice in the present study agree with the findings of Houssay et al. (1966) and Shugart (1980) with respect to melatonin injections. Although pinealectomy was performed on mature rats, several researchers have found no significant difference in testicular weights when compared to their controls (Motta et al., 1967; Kunkel, 1969; Debeljuk, 1969; Kinson and Robinson, 1970; Kinson and Peat, 1971; Kinson and Lui, 1973), but in contradiction to Debeljuk et al. (1971) who found that testicular weights of melatonin treated rats were lower than their controls. These researchers used a large dose of 750 ug melatonin/day/rat whereas in the present study and in others cited above the dose was in the range of 10-200 ug melatonin/day/animal. Turek (1977) did not observe testicular weight differences in pinealectomized male hamsters, however, Tamarkin et al. (1977) and Turek (1977) observed that melatonin administration to hamsters resulted in decreased testicular

weights. Gonadal weights in female rats and mice increased upon pinealectomy and decreased after melatonin or pineal extract treatment (Kitay and Altschule, 1954; Wurtman, 1961; Reiter, 1968). A sexual dimorphism obviously exists with respect to melatonin response.

A more reliable indication of testicular activity is the secretion of testosterone. Although no statistical analysis could be performed, there were no indications that testosterone levels were affected either by pinealectomy and/or melatonin injection. There was, however, a dramatic increase in testosterone levels as photoperiod increased, particularly between the 14L:10D and 24L:0D photoperiods. The present findings contradict those found by Shugart (1980) where long photoperiod mice had decreased testosterone levels. They do agree with those of Kinson and Peat (1971), however, who found that testosterone levels in rats increased with photoperiod.

Prostate gland size is indicative of testosterone activity. Pinealectomy was shown to be inhibitory and melatonin injections were stimulatory to the ventral prostate in the long and short photoperiods. Shugart (1980) found no differences in ventral prostate weights between melatonin injected mice and their controls.

The Shugart study, however, ran for a period of ninety days whereas the present study was performed over a period of sixty days. The possibility therefore exists that testosterone influenced changes of ventral prostate weights may have decreased over the thirty day period. Pinealectomy in male rats influenced increased prostate weights (Motta et al., 1967; Kinson and Peat, 1971) and melatonin injections inflenced decreases in prostate weights (Motta et al., 1967; Kinson and Robinson, 1970; Debeljuk et al., 1971; Kinson and Peat, 1971). Reiter et al. (1974) reported that the prostate weights of pinealectomized hamsters were larger than their controls. Melatonin may or may not influence changes in prostatic weights directly. As was stated previously, melatonin may influence testosterone secretion by way of the pituitary and, hence, mask any changes brought about by direct action of melatonin. In many studies pinealectomy was performed after puberty whereas in the present experiment the pineal was removed in sexually immature mice to prevent the influence of androgens on the prostates. Post-pubertal testosterone influence on prostatic tissue may account for some of the differences observed by researchers. Additionally, many researchers have reported both ventral and dorsal prostates as a single unit rather

than as two organs as was done in the present study. This would serve to mask individual changes in the two organs.

Pinealectomy had no effect on dorsal prostate weights in the present study, however, melatonin injections were inhibitory to dorsal prostate weights in sham mice and stimulatory in pinealectomized mice. This disagrees with the results of Shugart (1980) of no difference in dorsal prostate weights in melatonin injected mice and their controls. They do agree with results obtained in rats (Motta et al., 1967; Kinson and Robinson, 1970; Debeljuk et al., 1971; Kinson and Peat, 1971) that have shown that melatonin treated animals had smaller prostates than their controls. The above studies do not explain why melatonin would be stimulatory in pinealectomized mice but inhibitory in shams. The specific effect could very well depend on the circulating titers of melatonin in the blood as it appears that small amounts of melatonin (10 ug/day) would be sufficient to stimulate this accessory sex organ. In excess (normal titers + 10 ug/day) it would be inhibitory.

No abnormalities were found in testicular histology which is in agreement with the findings of other researchers in pinealectomized mice (Baum, 1968), melatonin injected mice (Shugart, 1980), melatonin

injected rats (Kunkel, 1969), and pinealectomized hamsters (Reiter, 1968). Although no comparable studies could be found in male mammals, Relkin (1972) did find that female rats reared in complete darkness proved to be fertile despite ovarian weight decrease. The present study found no indication that pinealectomy, melatonin injection, or photoperiod had any effect on the ability of male M. musculus to sire offspring.

In summary, none of the manipulations had any effect on fertility which throws doubt on the significance of the pineal for the reproductive competence of male laboratory mice. Ablation of the pineal gland and/or melatonin injections may have subtle, yet transient, effects on the organs examined in the present study. Under a short term study (three weeks or less) changes in organs may be observed but under a long term experiment, such as the present one, these changes may become less noticeable. Since the manipulations had no effect on the reproductive system of the male mouse, as a whole, it is safe to state that the pineal is not necessary for the reproductive capability of the male laboratory mouse, M. musculus, over a sixty day period.

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Table 1. Body weight increases of pinealectomized and sham operated male mice subjected to varied photoperiods and Melatonin injections (Mean ± S.D.) (Top figures are gram weight increases; Bottom figures are percent increase).

	PHOTOPERIOD		
	1.5L:22.5D	14L:10D	24L:0D
Pinealectomy			
Melatonin	14.9 ± 2.5 (67.2 ± 21.6)	16.6 ± 1.4 a (91.2 ± 5.1) a	18.5 ± 4.7 (93.4 ± 22.8)
Vehicle	15.4 ± 3.3 (79.1 ± 20.7)	18.4 ± 1.2 a,c (113.9 ± 9.7) a	15.2 ± 3.1 (76.9 ± 13.4)
Sham Operated			
Melatonin	16.9 ± 4.5 (86.9 ± 28.2)	17.3 ± 2.1 (97.0 ± 14.6)	18.5 ± 1.8 (89.8 ± 9.6)
Vehicle	17.6 ± 5.3 (90.5 ± 31.9)	20.2 ± 2.4 c (117.5 ± 26.0)	16.1 ± 2.7 (79.0 ± 10.6)

a = Pinealectomy-melatonin vs. Pinealectomy-vehicle ($p<0.05$)
c = Pinealectomy-vehicle vs. Sham-vehicle ($p<0.05$)

Table 2. Pituitary weights of pinealectomized and sham operated male mice subjected to varied photoperiods and Melatonin injections (Mean ± S.D.) (Top figures are milligram weights; Bottom figures are milligram percent body weight).

	PHOTOPERIOD		
	1.5L:22.5D	14L:10D	24L:0D
Pinealectomy			
Melatonin	1.3 ± 0.6 (4.0 ± 2.1) d	0.5 ± 0.4 a (1.4 ± 1.0) a,d	2.8 ± 0.1 a (7.3 ± 1.8)
Vehicle	1.4 ± 0.6 c (4.1 ± 1.8) c	1.6 ± 0.5 a,c (4.7 ± 1.6) a,c	1.4 ± 1.0 a,c (4.1 ± 3.3) c
Sham Operated			
Melatonin	2.2 ± 1.0 (5.9 ± 2.6) d	1.3 ± 0.4 b (3.6 ± 1.3) d	2.8 ± 1.1 (7.2 ± 3.0)
Vehicle	2.5 ± 0.6 c (7.5 ± 2.0) c	0.7 ± 0.4 b,c (2.1 ± 1.1) c	2.9 ± 0.5 c (7.7 ± 1.8) c

a = Pinealectomy-melatonin vs. Pinealectomy-vehicle ($p<0.05$)
b = Sham-melatonin vs. Sham-vehicle ($p<0.05$)
c = Pinealectomy-vehicle vs. Sham-vehicle ($p<0.05$)
d = Pinealectomy-melatonin vs. Sham-melatonin ($p<0.05$)

Table 3. Ventral prostate weights of pinealectomized and sham operated male mice subjected to varied photoperiods and Melatonin injection (Mean ± S.D.) (Top figures are milligram weights; Bottom figures are milligram percent body weights).

	PHOTOPERIOD		
	1.5L:22.5D	14L:10D	24L:0D
Pinealectomy			
Melatonin	6.1 ± 2.7	3.5 ± 1.6	5.5 ± 2.9 a
	(24.3 ± 8.3) a	(9.8 ± 4.5)	(15.2 ± 8.4) a
Vehicle	3.7 ± 1.5 c	2.7 ± 1.7	2.7 ± 0.9 a,c
	(10.7 ± 5.3) a,c	(7.7 ± 4.8)	(7.7 ± 2.5) a,c
Sham Operated			
Melatonin	6.8 ± 1.6	2.4 ± 1.4	4.0 ± 1.4
	(18.8 ± 5.3)	(6.9 ± 4.4)	(10.4 ± 3.5)
Vehicle	7.8 ± 2.4 c	2.6 ± 1.6	5.4 ± 2.9 c
	(20.4 ± 6.8) c	(7.0 ± 4.3)	(14.0 ± 6.7) c

a = Pinealectomy-melatonin vs. Pinealectomy-vehicle ($p<0.05$)
c = Pinealectomy-vehicle vs. Sham-vehicle ($p<0.05$)

Table 4. Dorsal prostate weights of pinealectomized and sham operated male mice subjected to varied photoperiods and Melatonin injections (Mean ± S.D.) (Top figures are milligram weights; Bottom figures are milligram percent body weights).

	PHOTOPERIOD		
	1.5L:22.5D	14L:10D	24L:0D
Pinealectomy			
Melatonin	5.5 ± 2.0 a	5.1 ± 1.4	8.7 ± 3.1 a
	(16.5 ± 7.0)	(14.7 ± 4.4)	(22.3 ± 5.6)
Vehicle	2.8 ± 0.8 a,c	3.9 ± 1.0	4.4 ± 3.0 a
	(7.9 ± 2.4) c	(11.5 ± 2.6)	(12.2 ± 8.1)
Sham Operated			
Melatonin	4.5 ± 2.5	3.7 ± 2.1	8.1 ± 3.8
	(11.9 ± 5.9)	(10.8 ± 7.1)	(20.9 ± 15.6) b
Vehicle	5.9 ± 2.5 c	4.4 ± 4.6	7.8 ± 3.8
	(15.6 ± 6.2) c	(11.5 ± 11.5)	(19.0 ± 11.6) b

a = Pinealectomy-melatonin vs. Pinealectomy-vehicle ($p<0.05$)
b = Sham-melatonin vs. Sham-vehicle ($p<0.05$)
c = Pinealectomy-vehicle vs. Sham-vehicle ($p<0.05$)

Table 5. Testis weights of pinealectomized and sham operated mice subjected to varied photoperiods and Melatonin injections (Mean ± S.D.) (Top figures are milligram weights; Bottom figures are milligram percent weights).

	PHOTOPERIOD		
	1.5L:22.5D	14L:10D	24L:0D
Pinealectomy			
Melatonin	217.3 ± 22.1	258.3 ± 26.6	239.1 ± 49.5 d
	(644.4 ± 101.9)	(744.3 ± 81.4)	(622.2 ± 6.5)
Vehicle	238.8 ± 30.5	221.3 ± 43.6	239.3 ± 20.0
	(678.4 ± 56.8)	(638.2 ± 114.9)	(682.1 ± 105.3)
Sham Operated			
Melatonin	218.6 ± 42.9	245.7 ± 32.2	268.8 ± 35.7 d
	(598.8 ± 9.5)	(702.2 ± 139.5)	(683.7 ± 55.8)
Vehicle	255.7 ± 28.2	223.6 ± 15.8	245.6 ± 26.7
	(694.2 ± 143.1)	(594.7 ± 50.5)	(653.0 ± 79.1)

d = Pinealectomy-melatonin vs. Sham-melatonin ($p<0.05$)

Table 6. Testosterone levels of pooled plasma from pinealectomized and sham operated mice subjected to varied photoperiods and Melatonin injection (Expressed as ug %).

	PHOTOPERIOD		
	1.5L:22.5D	14L:10D	24L:0D
Pinealectomy			
Melatonin	0.04	0.06	0.36
Vehicle	0.03	0.04	0.24
Sham Operated			
Melatonin	0.07	0.15	0.39
Vehicle	0.10	0.20	0.45

Table 7. Average number of progeny of pinealectomized and sham operated male mice subjected to various photoperiods and Melatonin injection after a single mating.

	PHOTOPERIOD		
	1.5L:22.5D	14L:10D	24L:0D
Pinealectomy			
Melatonin	7.5	12.5	10
Vehicle	11.5	11	10**
Sham Operated			
Melatonin	11*	11.5	10.5
Vehicle	11*	11.5	11

* Progeny of one male-female pair; other female ate some of her young and was not included.

** Progeny of one male-female pair; other female did not become pregnant.

Fig. 1. Comparison of mean increase in body weight in grams among experimental mouse groups against photoperiod.

Legend:

▲ = Pinealectomy-melatonin

△ = Pinealectomy-vehicle

■ = Sham-melatonin

□ = Sham-vehicle

Photoperiod:

1.5 = 1.5L:22.5D

14 = 14L:10D

24 = 24L:0D

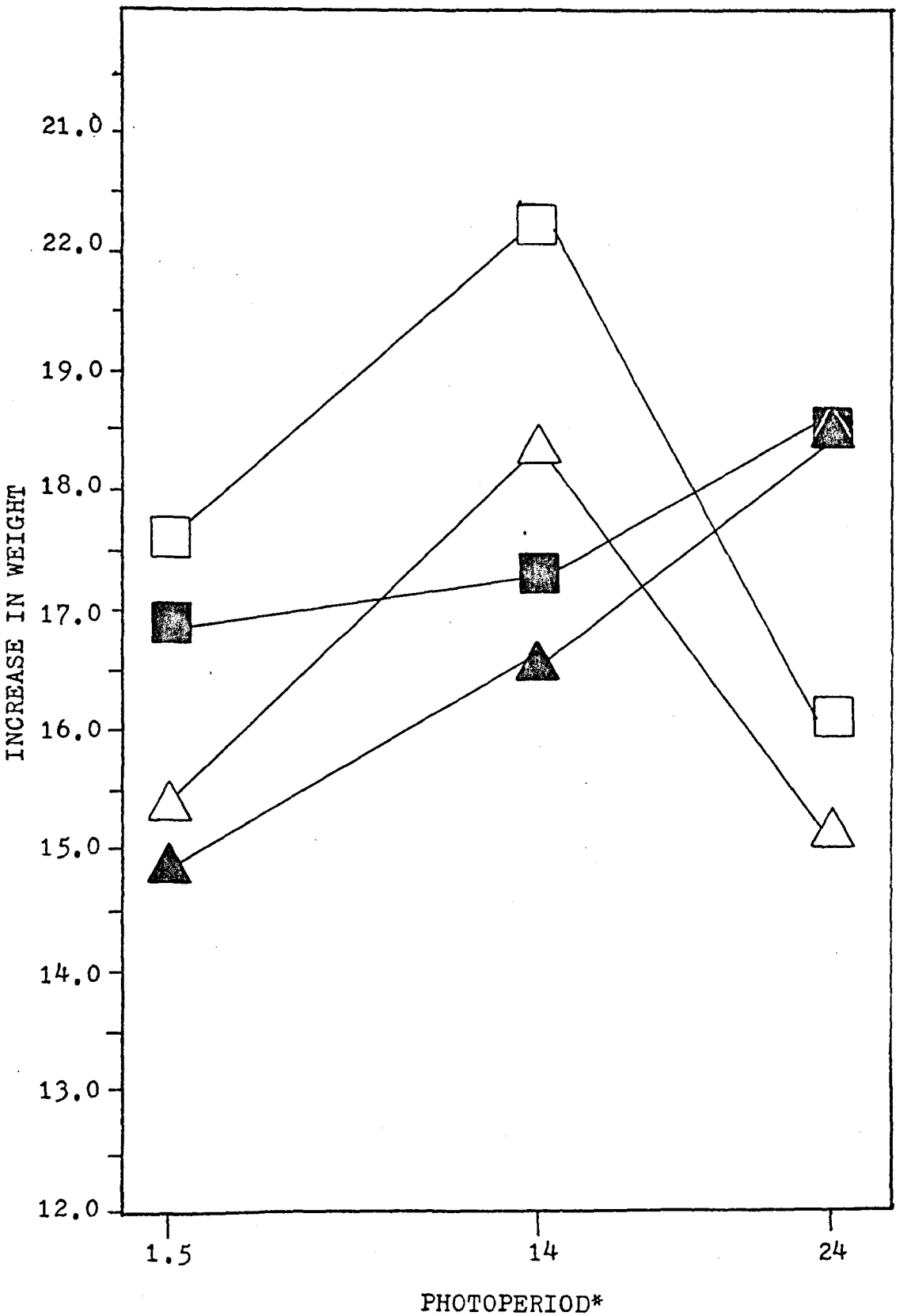

21.0
22.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
INCREASE IN WEIGHT
1.5
14
24
PHOTOPERIOD*

Fig. 2. Comparison of mean increase in body weights expressed as percent increase among experimental mouse groups against photoperiod.

* see Legend, Fig. 1.

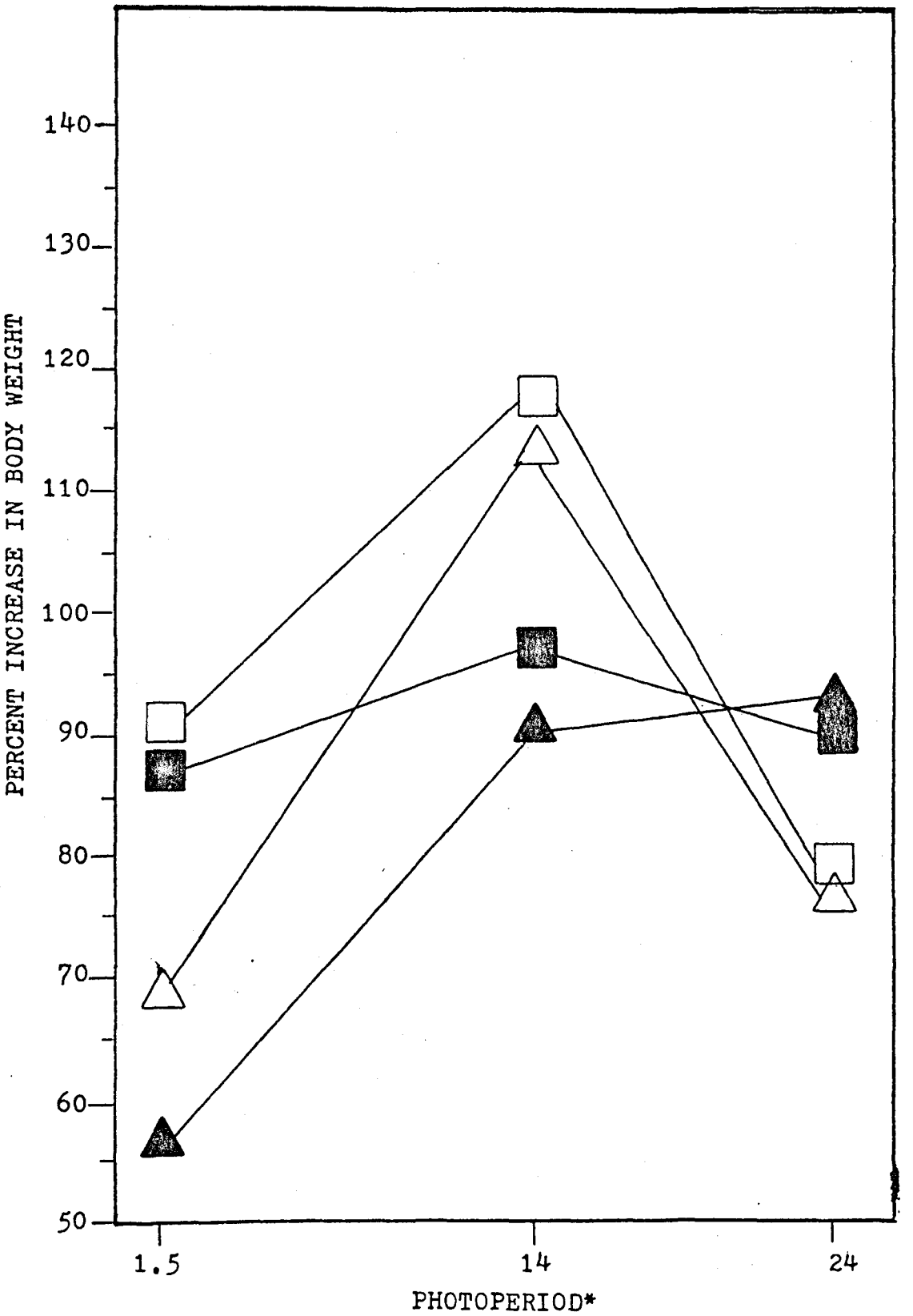

PERCENT INCREASE IN BODY WEIGHT
140
130
120
110
100
90
80
70
60
50
1.5
14
24
PHOTOPERIOD*

Fig. 3. Comparison of mean pituitary weights expressed in milligrams among experimental mouse groups against photoperiod.

* see Legend, Fig. 1.

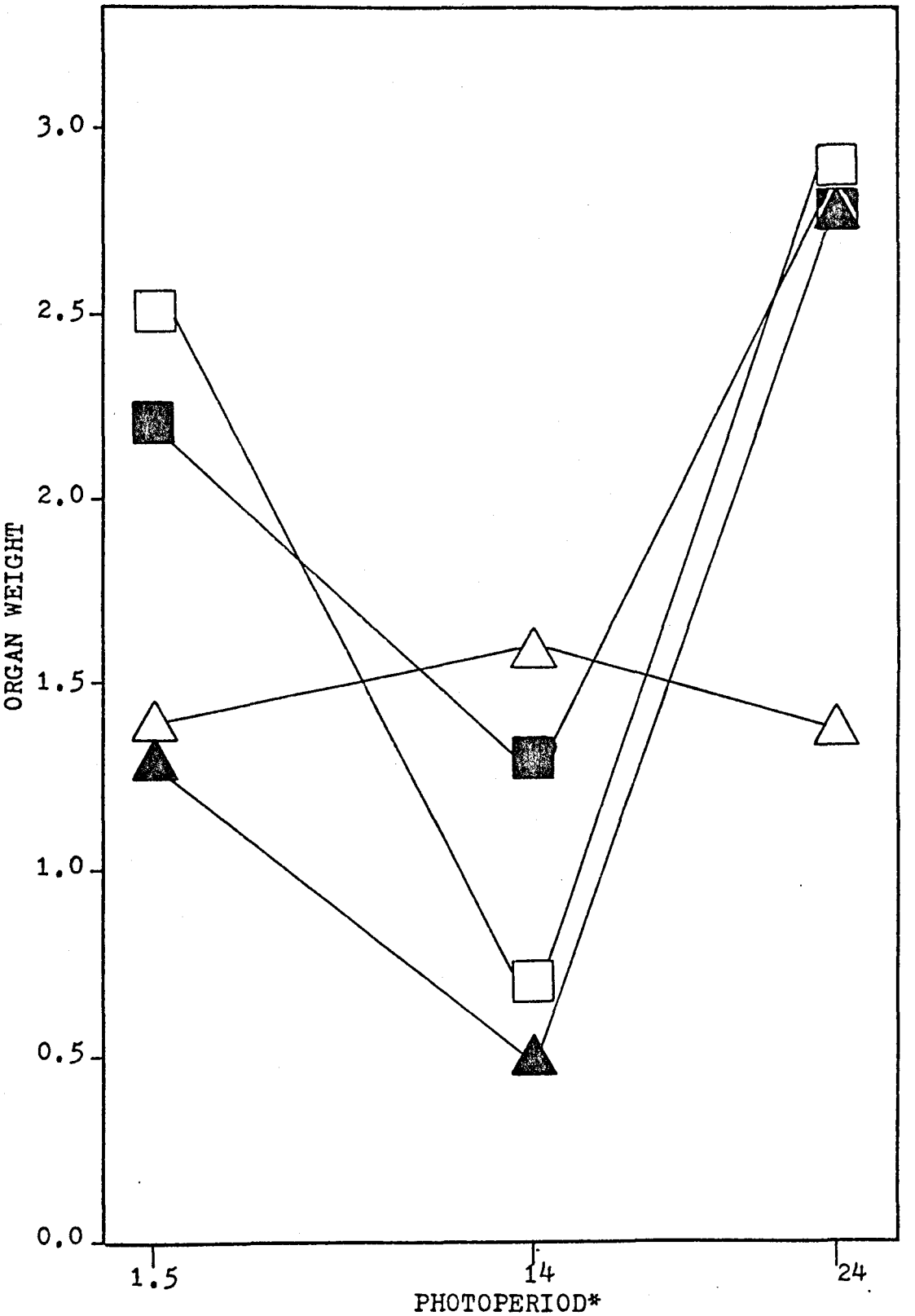

3.0
2.5
2.0
1.5
1.0
0.5
0.0
ORGAN WEIGHT
1.5
14
24
PHOTOPERIOD*

Fig. 4. Comparison of mean pituitary weights expressed as percent body weight among experimental mouse groups against photoperiod

* see Legend, Fig. 1.

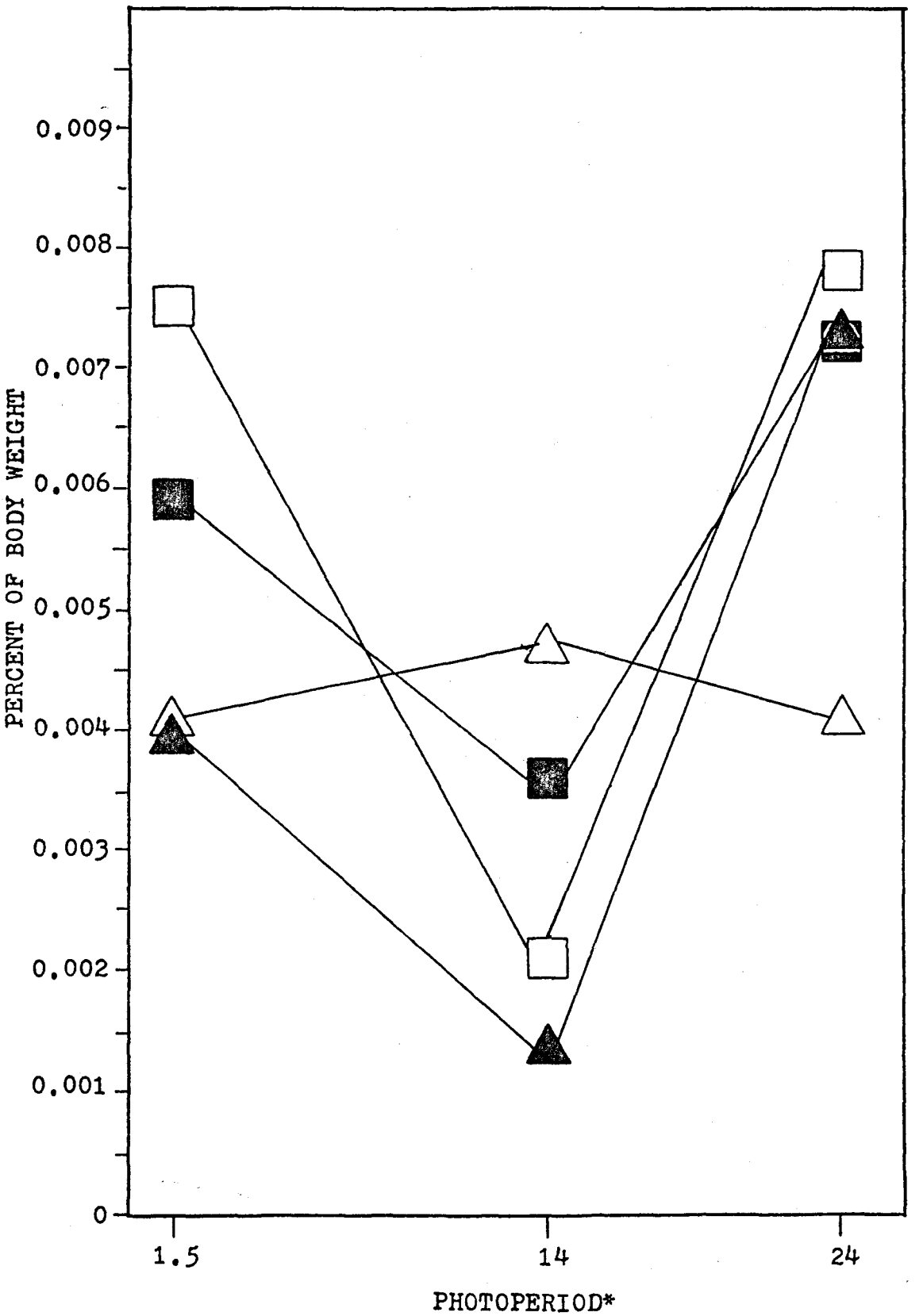

0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
PERCENT OF BODY WEIGHT
1.5
14
24
PHOTOPERIOD*

Fig. 5. Comparison of mean ventral prostate weights expressed as milligrams among experimental mouse groups against photoperiod.

* see Legend, Fig. 1.

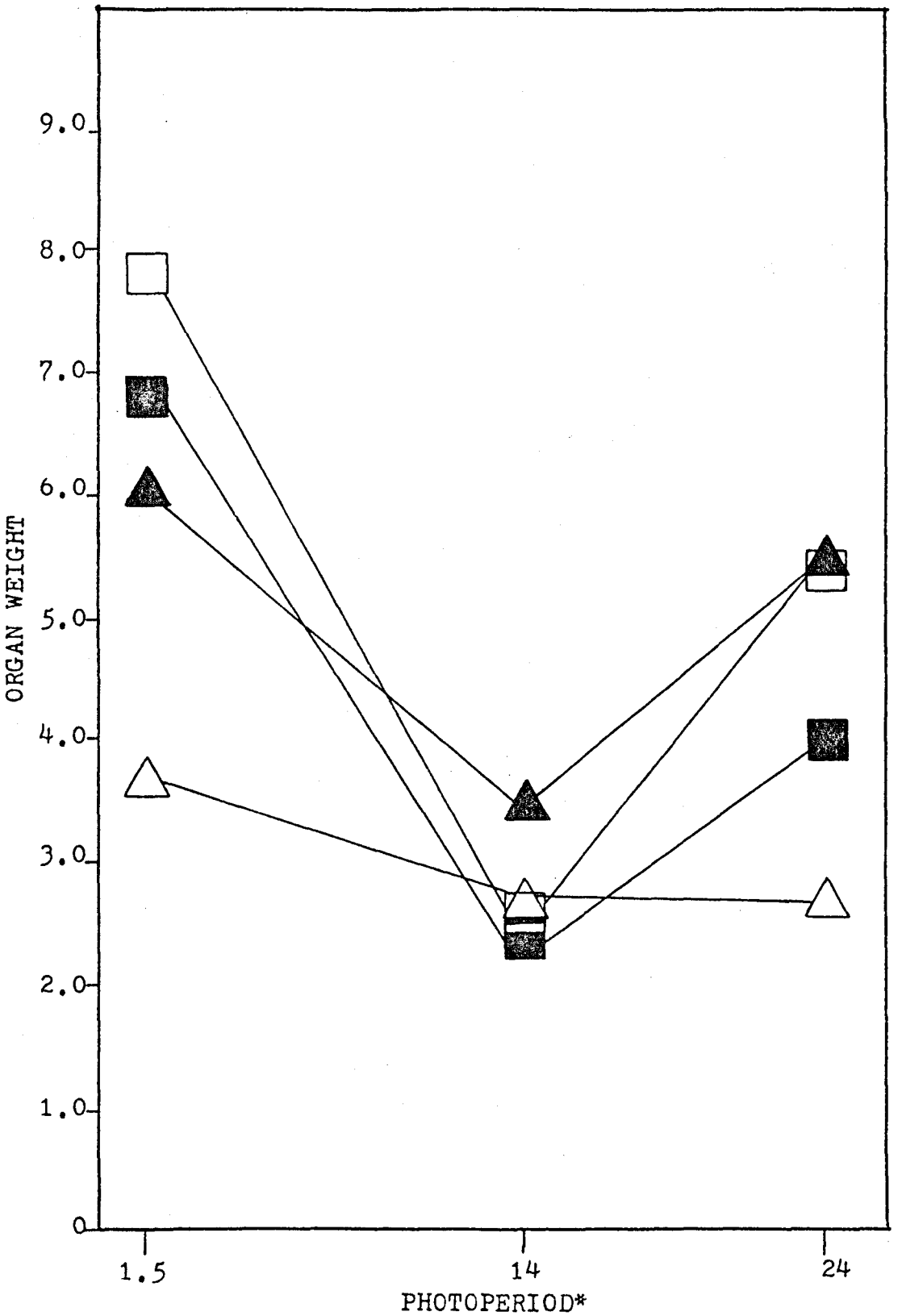

9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
ORGAN WEIGHT
1.5
14
24
PHOTOPERIOD*

Fig. 6. Comparison of mean ventral prostate weights expressed as percent body weight among experimental mouse groups against photoperiod. * see Legend, Fig. 1.

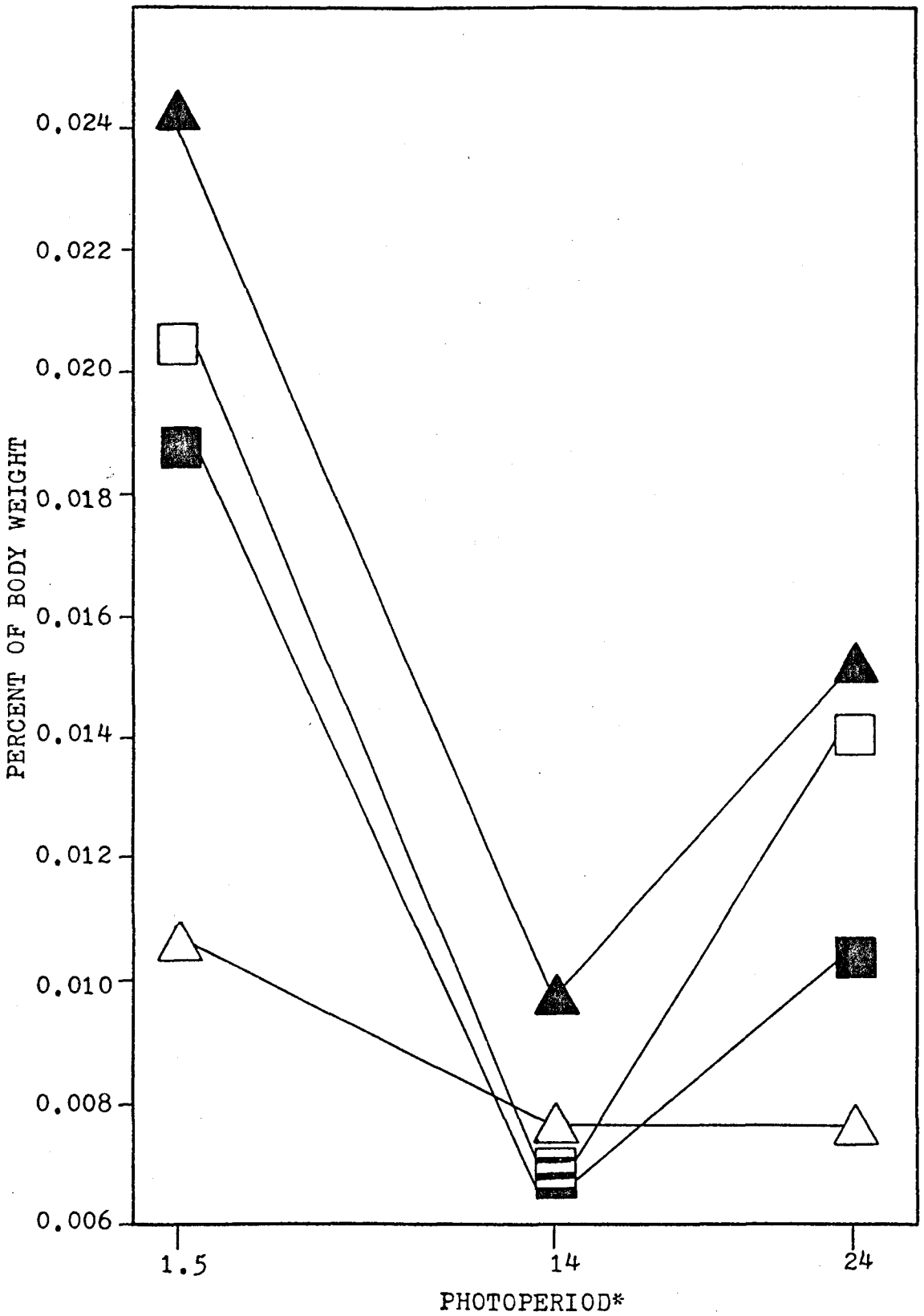

0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
PERCENT OF BODY WEIGHT
1.5
14
24
PHOTOPERIOD*

Fig. 7. Comparison of mean dorsal prostate weights expressed as milligrams among experimental mouse groups against photoperiod.

* see Legend, Fig. 1.

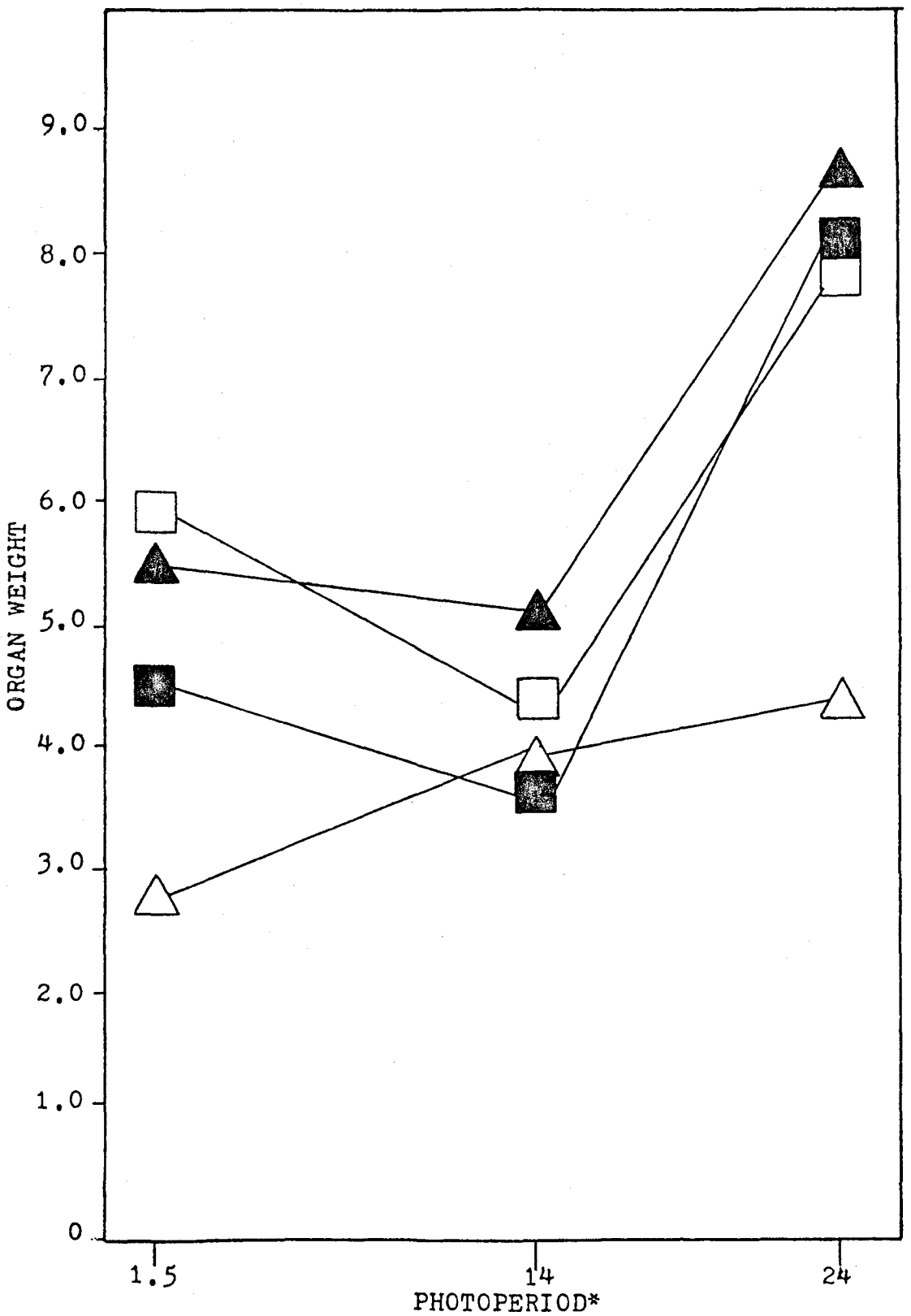

9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
ORGAN WEIGHT
1.5
14
24
PHOTOPERIOD*

Fig. 8. Comparison of mean dorsal prostate weights expressed as percent body weight among experimental mouse groups against photoperiod.
* see Legend, Fig. 1.

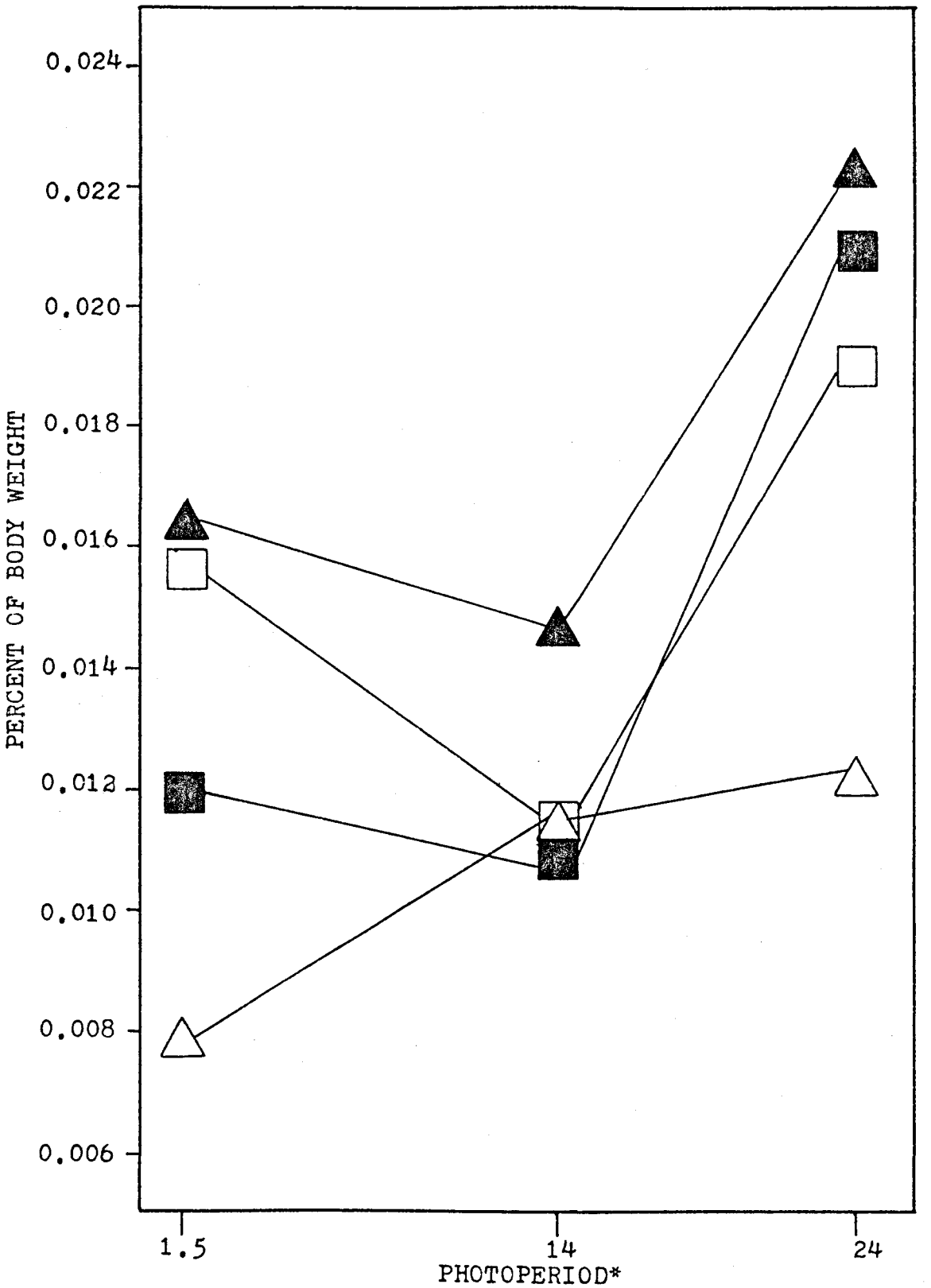

0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
PERCENT OF BODY WEIGHT
1.5
14
24
PHOTOPERIOD*

Fig. 9. Comparison of mean testis weights expressed as grams among experimental mouse groups against photoperiod.

* see Legend, Fig. 1.

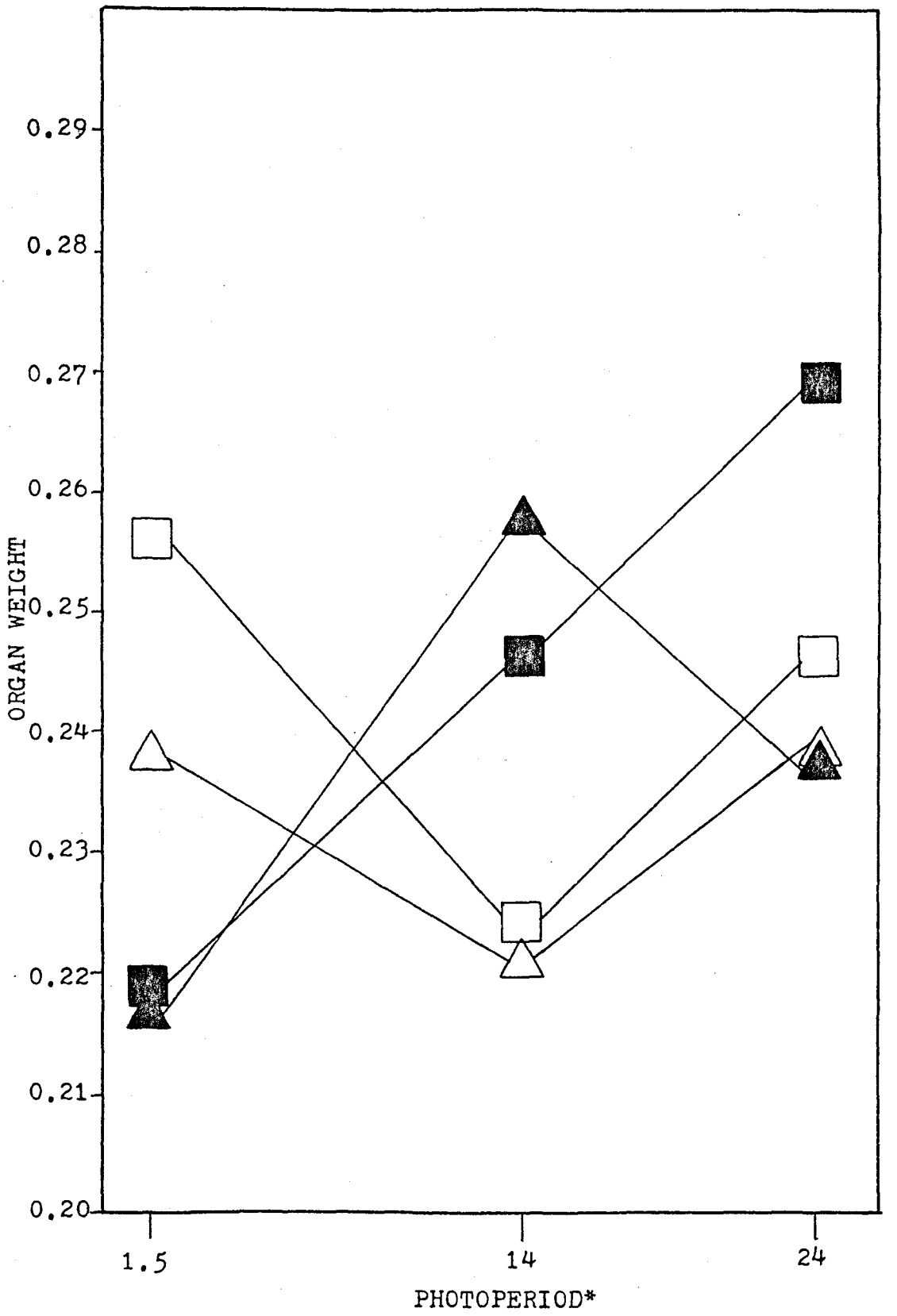

0.29
0.28
0.27
0.26
0.25
0.24
0.23
0.22
0.21
0.20
ORGAN WEIGHT
1.5
14
24
PHOTOPERIOD*

Fig. 10. Comparison of mean testis weights expressed as percent body weight among experimental mouse groups against photoperiod.

* see Legend, Fig. 1.

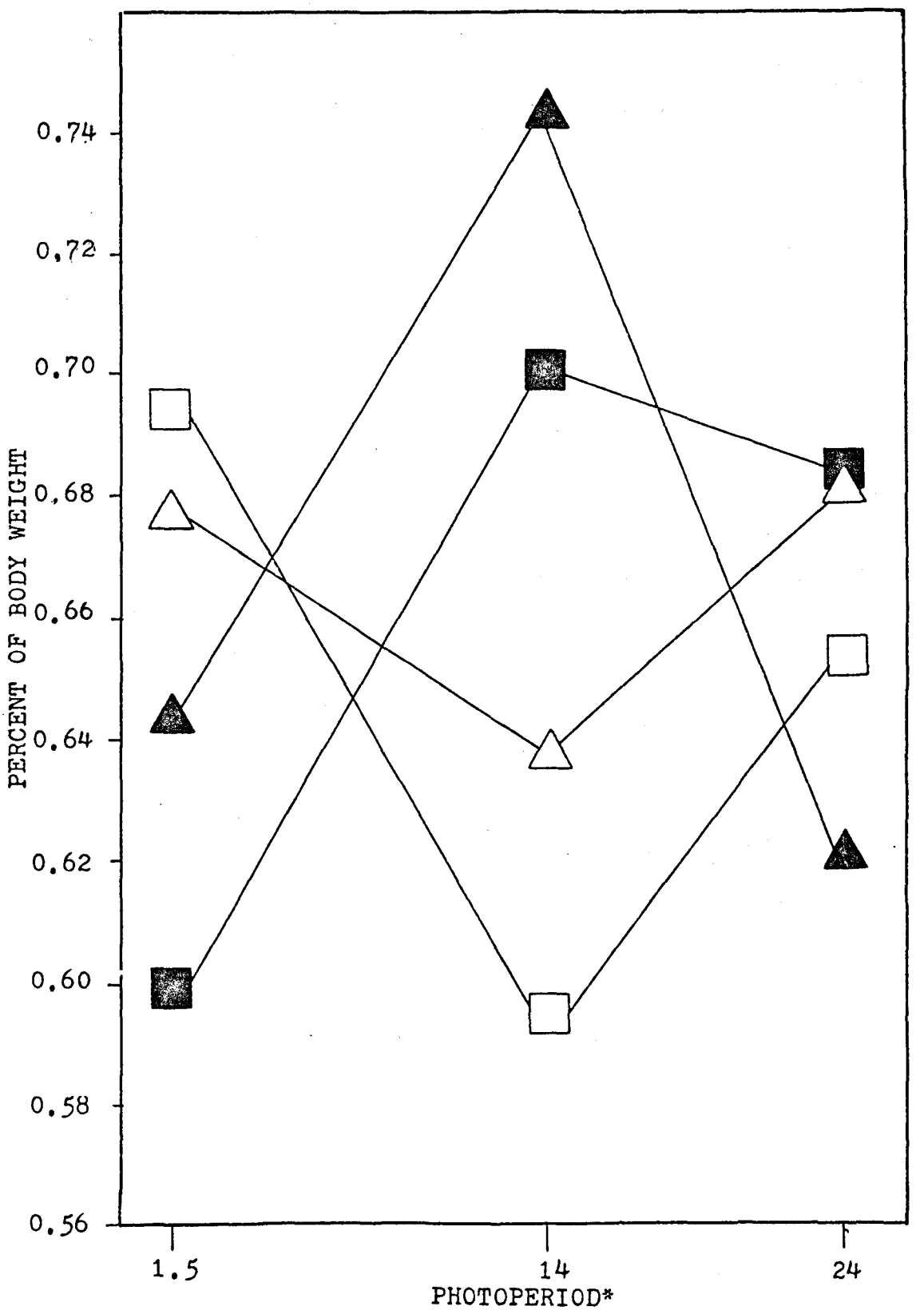

0.74
0.72
0.70
0.68
0.66
0.64
0.62
0.60
0.58
0.56
PERCENT OF BODY WEIGHT
1.5
14
24
PHOTOPERIOD*

Fig. 11. Comparison of plasma testosterone levels expressed as micrograms percent among experimental mouse groups against photoperiod.
* see Legend, Fig. 1.

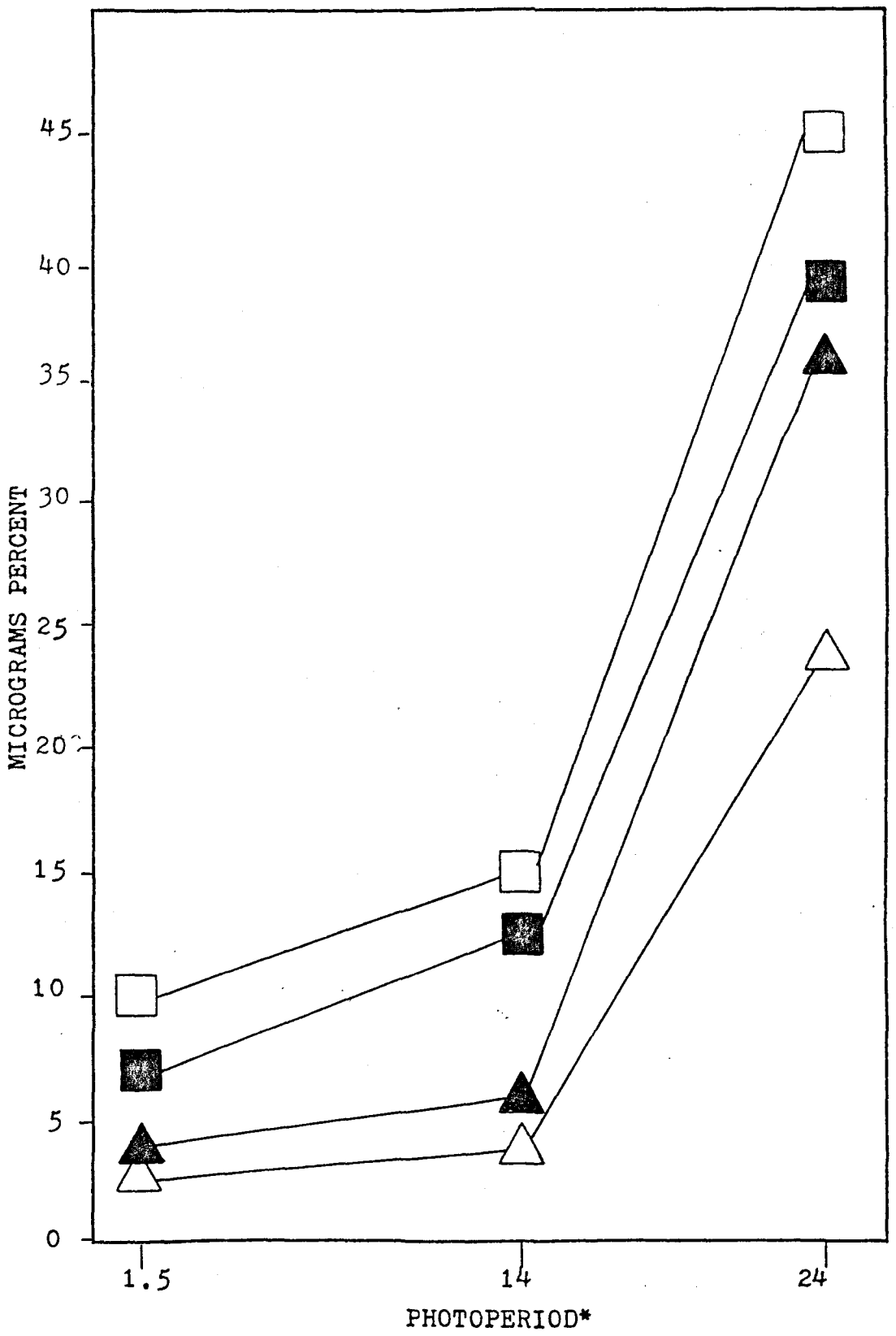

45
40
35
30
25
20
15
10
5
0
MICROGRAMS PERCENT
1.5
14
24
PHOTOPERIOD*

Fig. 12. Photomicrographs of testis sections of mice exposed to a 1.5L:22.5D photoperiod (450x).

1. Pinealectomy-vehicle
2. Pinealectomy-melatonin
3. Sham-vehicle
4. Sham melatonin

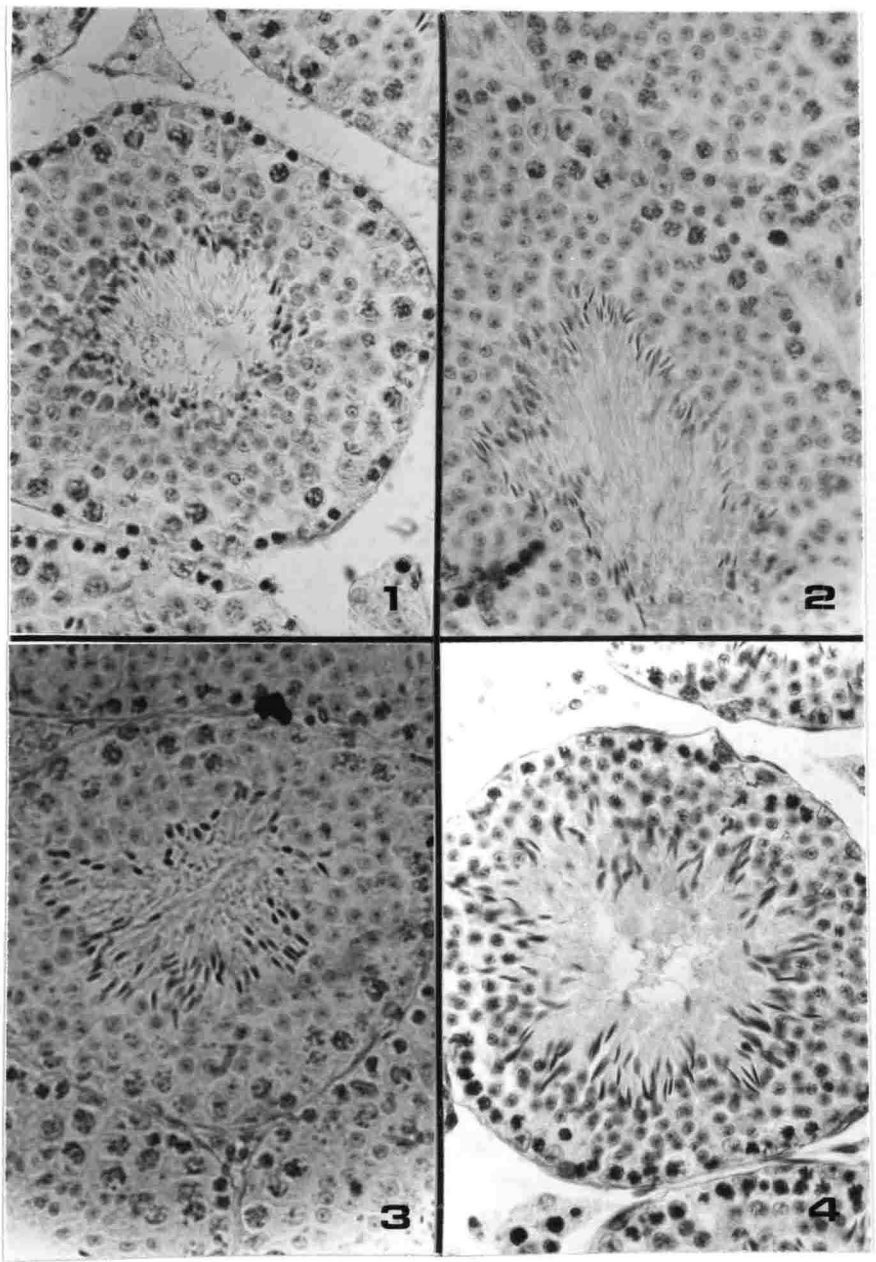

1
2
3
4

Fig. 13. Photomicrographs of testis sections of mice exposed to a 14L:10D photoperiod (450x).

1. Pinealectomy-vehicle
2. Pinealectomy-melatonin
3. Sham-vehicle
4. Sham-melatonin

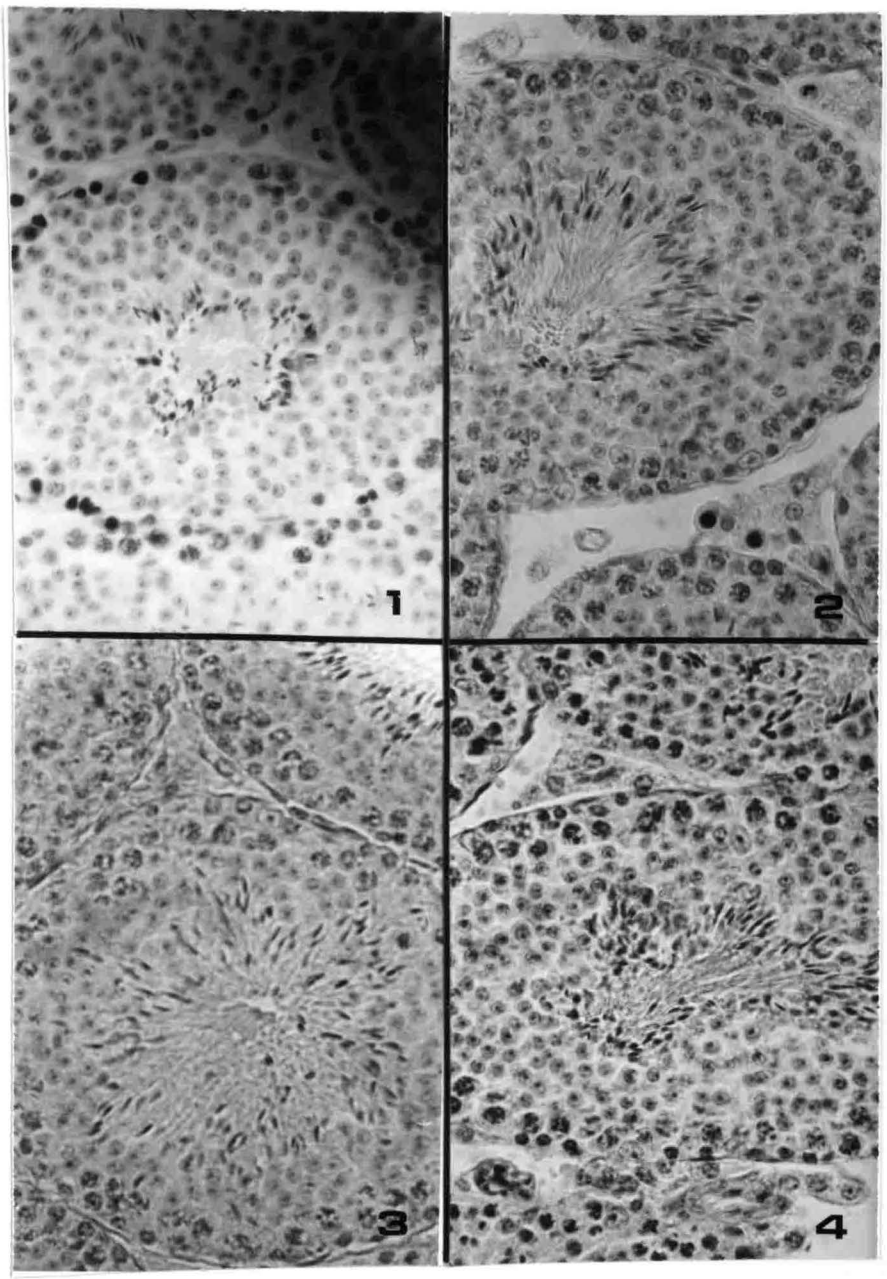
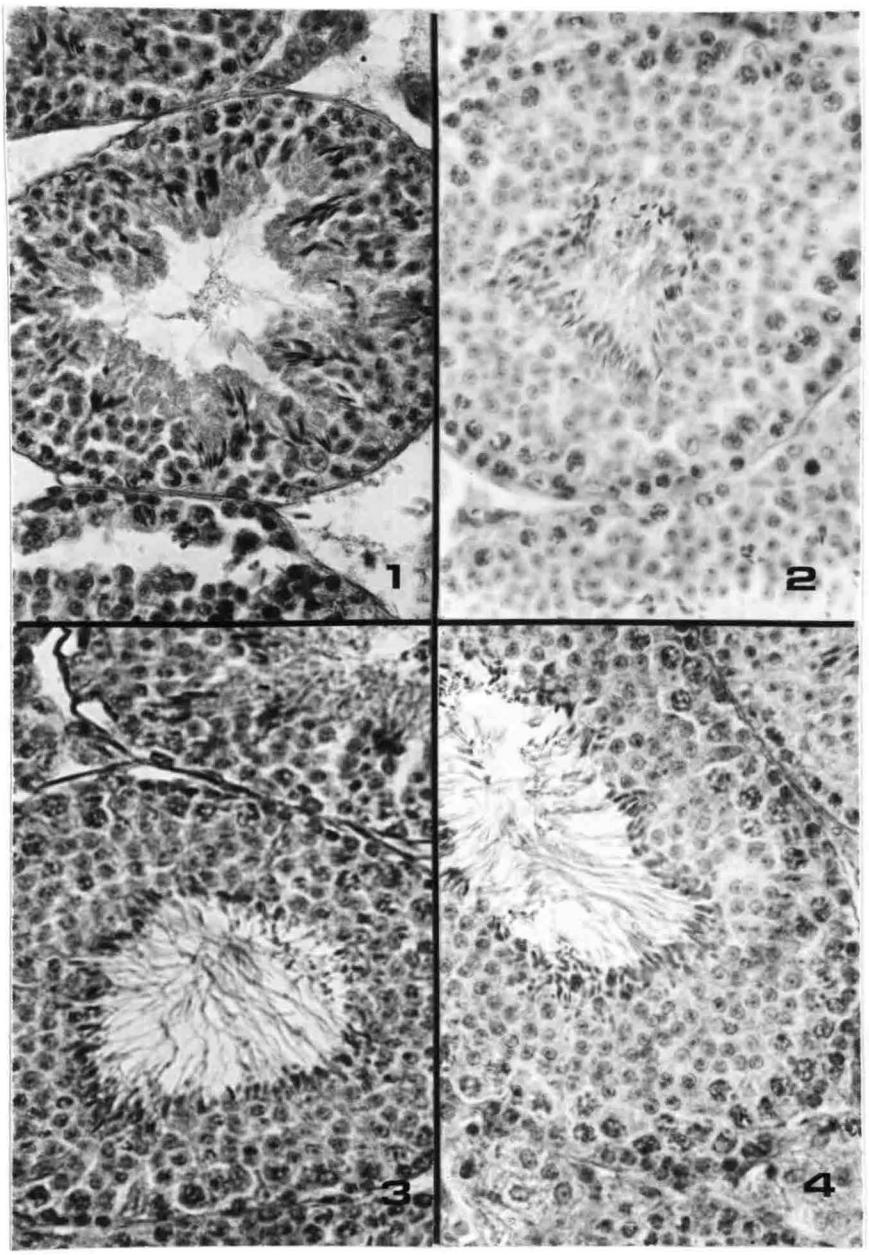

1
2
3
4

Fig. 14. Photomicrographs of testis sections of mice exposed to a 24L:0D photoperiod (450x).

1. Pinealectomy-vehicle
2. Pinealectomy-melatonin
3. Sham-vehicle
4. Sham-melatonin


1
2
3
4

VITA

John Ernest Constantine was born on 13 August 1957 in Richmond, Virginia. He received his elementary and secondary school education in the Richmond Public School system graduating from Thomas Jefferson High School in June 1975. He attended the University of Richmond and received the Bachelor of Science degree in Biology in May 1979. He continued his studies at the University receiving the Master of Science degree in Biology in August 1981.

While at the University of Richmond he was elected to membership in Phi Eta Sigma Freshman Honor Society, serving as treasurer in 1976-1977. He was inducted into Beta Beta Beta Biological Honor Society in October 1977 and served as historian in 1978-1979. In March 1981 he was made an associate member of the Society of the Sigma Xi, a national research organization.

He will enter the Ph.D. program in Pharmacology at the Medical College of Virginia of Virginia Commonwealth University in August 1981.